

**Title: Prevalence and risk factors for betaherpesvirus DNAemia in infants aged between 3 weeks and 2 years of age, admitted to a large referral hospital in sub-Saharan Africa**

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40-word summary: Study describing the prevalence of betaherpesvirus DNAemia among a broad cross-section of hospitalized African infants aged 3 weeks to 2 years, showing independent associations between cytomegalovirus infection and HIV, being underweight and an admissions diagnosis of meningitis.

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## ABSTRACT

**Background:** Betaherpesviruses are established causes of morbidity and mortality in immune suppressed patient groups but they have been little studied in sub-Saharan Africa, the epicenter of the HIV pandemic. In this region, primary infections with human cytomegalovirus (HCMV) and human herpesvirus 6 (HHV-6) are endemic in infancy, but the clinical impact of these infections among pediatric inpatient groups is poorly characterized and assumptive, based largely on data from Western populations.

**Methods:** We used Taqman PCR to screen sera from a group of 303 paediatric inpatients aged between 3 weeks and 2 years of age, at the University Teaching Hospital, Lusaka, Zambia. We report the prevalence of DNAemia and viral loads within this patient group, and evaluate possible clinical associations/risk factors for betaherpesvirus infections in these hospitalized infants.

**Results:** We detected betaherpesvirus DNAemia in 59.1% (179/303) of infants. HCMV was the most prevalent (41.3%), followed by HHV-6B (20.5%), HHV-7 (20.1%) and HHV-6A (0.3%). HIV infection (OR2.31, 95%CI:1.37-3.9,  $p=0.002$ ) being underweight (OR1.82, 95%CI:1.06-3.12,  $p=0.03$ ) and an admission diagnosis of suspected meningitis (OR5.72, 95%CI1.07-30.5,  $p=0.041$ ) were independently associated with an increased odds of HCMV DNAemia. Conversely, HHV-6B and HHV-7 DNAemia were not associated with HIV, underweight or admission diagnosis. Median HCMV viral load was moderately but significantly higher in HIV-infected children.

**Conclusions:** Highly prevalent HCMV DNAemia was independently associated with HIV infection and being underweight across all age groups, and was also associated with meningitis, with previously under appreciated implications for the health and development of African children.

**KEYWORDS:** Betaherpesvirus, HCMV, HHV-6, HHV-7, cytomegalovirus, malnutrition, underweight, HIV, Africa, infant

## INTRODUCTION

The human betaherpesviruses (Human cytomegalovirus (HCMV), Human herpesvirus 6A (HHV-6A), Human herpesvirus 6B (HHV-6B) and Human herpesvirus 7 (HHV-7)) are important causes of morbidity in infants and HCMV and HHV-6 are well-established causes of morbidity and mortality in immune-compromised patients [1, 2], but they have been little studied in sub-Saharan Africa, the WHO region with the highest pediatric disease burden and also the epicenter of the HIV pandemic. Primary HCMV and HHV-6 infections are endemic in African infants [3-5], establishing life-long latency with periodic reactivations or re-infections [1]. The great challenge in herpes virology is to differentiate active infections causing pathology from background sub-clinical viraemia. To this end, the latest quantitative molecular diagnostics are being used to establish clinically informative viral load cut-offs in different patient groups and within different compartments, which can inform on treatment [6-8]. The betaherpesviruses are highly cell-associated and within vulnerable patient groups detection of high HCMV and HHV-6 DNA loads in serum is indicative of active infections linked with poor outcomes [8, 9], but absence or low viral loads in serum does not rule out localized infection restricted to specific compartments [10].

Recent prospective studies and careful review of some older but overlooked studies, has led to a growing awareness of the importance of HCMV infections in high seroprevalence settings [11-13], as a major cause of mortality in HIV-infected children [14, 15] and as a cause of developmental delay [5] and hearing loss [16-18] in children through either congenital or early infant infections, with maternal HIV infection and lower CD4 count associated with higher prevalence of congenital infection [15, 19]. Human herpesvirus 6A (HHV-6A), Human herpesvirus 6B (HHV-6B) and Human herpesvirus 7 (HHV-7)) have been less well studied in sub-Saharan Africa. HHV-6B primary infection (and to a lesser extent HHV-7), is the etiological agent of *exanthema subitum*, an endemic childhood illness characterized by fever, diarrhea and skin rash [20, 21], and is strongly associated with febrile seizures [22] including febrile status epilepticus (FSE) [23]. HHV-6A is rarely found in children in Europe, the U.S and Japan, but a recent study found HHV-6A to be endemic in healthy Zambian infants [3]. Elsewhere, HHV-6A is more common in immune-compromised adults, and recent animal model studies have suggested possible roles for HHV-6A in immune deficiency [24] and AIDS progression [25]. A study in U.S children also demonstrated an association between HHV-6B and AIDS progression [26].

In sub-Saharan Africa tertiary referral hospitals concentrate large numbers of seriously ill children from impoverished urban communities. At such centers drugs to control HIV, TB, Malaria and other common bacterial, fungal and parasite infections are broadly available, yet mortality rates remain high [27]. Rapid

diagnostics and treatment for viral infections are broadly unavailable but HCMV is an important cause of respiratory disease in HIV-infected African children [8, 14, 28, 29] and HHV-6 and HHV-7 are possible causes of non-malarial CNS infections [30]. HCMV pneumonia can be treated successfully with intravenous Ganciclovir in both immunocompromised (HIV-infected South African infants with confirmed HCMV infection)[28, 29] and immunocompetent children [31]. There are generic anti-CMV drugs in the pipeline and anti-betaherpesvirus activity has been reported for several low cost alternatives such as artesunate [32, 33] and the immune stimulant ‘active hexose correlated compound’ (AHCC)[34]. Here we present data from a broad group of admitted infants at the University Teaching Hospital in Lusaka, Zambia, with the aim of determining prevalence of betaherpesvirus DNAemia and viral loads, and identifying possible clinical associations and risk factors.

## **METHODS**

### **Ethics approval**

The study was approved by the Biomedical Research Ethics Committee of the University of Zambia School of Medicine (UNZABREC), Lusaka, Zambia. The mothers/guardians of all participants gave written informed consent.

### **Study Design and Setting**

We conducted a retrospective observational study to determine the prevalence, viral loads and risk factors associated with betaherpesvirus DNAemia in admitted infants at the University Teaching Hospital, Lusaka, Zambia. We selected biobanked sera specimens from infants aged between 3 weeks and 2 years of age, admitted to the general paediatric inpatient wards, who had taken part in a TB diagnostic trial [35], and screened them for all four betaherpesviruses and measured viral loads. The degree to which the recruited children were representative of the broader inpatient population was evaluated by comparing key demographic data with that from general admission records.

### **DNA Extraction & PCR Analysis**

Laboratory analysis was conducted in our dedicated 3-room molecular diagnostics laboratory. DNA from serum was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) as previously described [4, 15]. Extraction controls were included with every 11 samples. DNA extraction quality was monitored on every 11th sample using a Nanodrop (Thermo Fisher Scientific, Waltham, MA). Samples were tested for presence of HCMV, HHV-6A, HHV-6B and HHV-7 using three different quantitative Real Time Taqman

PCR assays as described [7, 36, 37]. Oligonucleotide sequences and thermocycling conditions were as indicated (Table 1). Real Time PCR was undertaken on a Rotor-Gene™ 6000 (Qiagen, Hilden, Germany). The fidelity of the PCR enzyme and purity of the DNA-extraction was controlled through amplification of the house-keeping gene,  $\beta$ -Actin, from every 11th sample. Positive (commercially produced standards), negative (molecular grade water), reagent (no template) and extraction controls were included with each run. HCMV genomic DNA was purchased from the National Institute for Biological Standards and Control (NIBSC, Potters Bar, U.K). Genomic DNA for HHV-6A, HHV-6B and HHV-7 were donated from the reagent repository of the HHV-6 Foundation (Santa Barbara, CA, U.S). Standard curves for quantitation of betaherpesvirus viral loads were prepared as follows: HCMV: lyophilized standard was re-suspended in elution buffer according to manufacturer's instructions to create a stock solution of  $5 \times 10^6$  copies/ml. HHV-6A, HHV-6B and HHV-7 standards were human sera containing  $5 \times 10^6$  copies/ml. 200 $\mu$ l was extracted and reconstituted in 200 $\mu$ l elution buffer. For all four viruses 10-fold serial dilutions were made down to  $5 \times 10^{-1}$  copies/ml. 5 $\mu$ l of each dilution (25,000, 2500, 250, 25 and 2.5 absolute copies) were then amplified in triplicate. The manufacturer's software was then used to generate standard curves against which Ct values of clinical specimens were converted to 'copies/ $\mu$ l extracted DNA'. Extracted DNA from clinical specimens was quantified (ng/ $\mu$ l) using a Nanodrop (Thermo Scientific, Waltham, MA, U.S) and 'viral genome equivalents/ $\mu$ g extracted DNA' calculated.

### **Statistical Analysis**

Data analysis was undertaken using SPSS version 21 (IBM, Armonk, NY, USA). Binary and continuous variables were compared by Pearson chi-squared test and Mann Whitney U respectively, between study groups and the general admitted population (Table 2). For under- or over-represented covariates, weighting variables were calculated using the formula 'prevalence in hospital population/prevalence in sample' and then multiplied together and divided by the mean weight to make a composite weighting variable. This composite weight was then used to estimate hospital population-weighted prevalences for each of the betaherpesviruses. Multivariate binary logistic regression analysis (on non-weighted data) was used to evaluate possible associations between various clinical and demographic factors and betaherpesvirus infection and virus loads. The Wald-Wolfowitz Runs test was used to evaluate the randomness of the distribution of positive samples. Median viral loads were compared by HIV status and underweight status using the Mann Whitney U test.

### **RESULTS**

## **Patient Recruitment**

We screened DNA-extracted sera from 303 infants aged between 3 weeks and 2 years of age. 42.5% of the infants were female, median age was 12 months (IQR 7-17), 34.8% of infants were HIV-infected and 36.3% of infants were considered to be malnourished on admission (Admitted to the malnutrition ward due to weight-for-height z-score < -2 or on clinical grounds at the discretion of the attending physician). Comparison with this routine admissions data showed that whilst gender was representative, the study infants were significantly older, more likely to be HIV infected and more likely to be malnourished (Table 2), consistent with suspicion of TB or TB risk factors (HIV infection, household contact etc..), which were key inclusion criteria on the parent trial [35].

## **Prevalence of betaherpesvirus infections**

Betaherpesvirus DNAemia was detected by PCR in sera from 59.1% (179/303) of infants (Table 3). All negative controls were negative and positive specimens were randomly distributed (analysis not shown). HCMV was the most prevalent virus (41.3%, 125/303), significantly more prevalent than HHV-6B (20.5%, 62/303)( $p < 0.001$ ) and HHV-7 (20.1% (61/303)( $p < 0.001$ ). Weighting for the over-representation of HIV infected children, older median age and increased prevalence of malnutrition, did not significantly effect betaherpesvirus prevalence (Table 3). HHV-6A was rare, with just one case: a male baby of 9 months of age, admitted with suspected pneumonia, TB or pertussis. A gastric aspirate was culture negative for TB, the baby was HIV negative and had a weight-for-age z-score of -1.22. The mother reported recent loss of appetite and weight loss, fevers, productive cough, difficulties breathing, and lethargy. This baby was also positive for HCMV and HHV-7, one of just 9 babies positive for 3 betaherpesviruses. 52 cases were co-infected with two viruses (Table 3).

## **Risk factors for betaherpesvirus infections**

HCMV DNAemia was significantly more prevalent among HIV-infected infants, than among HIV-uninfected infants (54.5% (55/101) vs 34.0% (65/191),  $p = 0.001$ , a univariate effect not seen for HHV-6B or HHV-7 (Table 3). Multivariate binary logistic regression analysis demonstrated that HCMV DNAemia in infants was independently associated with HIV infection (OR 2.31, 95%CI: 1.37-3.90,  $p = 0.002$ ) and being underweight (OR 1.82, 95%CI: 1.06-3.12,  $p = 0.03$ )(Table 4). Among 8 children admitted with suspected meningitis, 6 were positive for HCMV DNAemia (OR 5.72 [1.07-30.5],  $p = 0.041$ ). In our study group betaherpesvirus DNAemia was not associated with fever ( $\geq 38^{\circ}\text{C}$ ), breast-feeding (an established route

of transmission for HCMV, although only 13 infants were not being, or had not been, breast fed) or reported recent rash (none of the infants presented with a rash)(Table 4).

The odds of HCMV DNAemia decreased significantly with age (OR 0.95 95%CI: 0.91-0.99,  $p = 0.008$ ), with a similar trend for HHV-6B. Conversely, there was a trend for increased odds of HHV-7 DNAemia with age. To investigate these age trends in more detail, we plotted betaherpesvirus DNAemia prevalence by age in months (Figure 1a). We observed peaks of HCMV prevalence at 3-5 months, and 18-20 months, indicative of early primary infection (with some residual DNAemia from congenital infections) followed by reactivation/re-infection in older infants. A higher prevalence of HCMV DNAemia was associated with HIV infection (Figure 1b) and being underweight (Figure 1c) across all age groups.

HHV-6B displayed a similar temporal profile to HCMV, with peaks suggestive of primary and secondary infections (figure 1a). The temporal signature of HHV-7 DNAemia was clearly distinct from HCMV and HHV-6B, dropping from 20% to 10% over the first 6 months of life, but then steadily increasing back to 20% by 12 months, and remaining stable (Figure 1a). Co-infections with more than one betaherpesvirus were common, detected in 33.5% (60/179) of all cases (51 cases with two viruses, and 9 cases with 3 viruses) but HIV infected children were no more likely to have a double or triple infection than HIV uninfected children (data not shown). There was a significant correlation between HCMV and HHV-6B (Pearson correlation 0.157,  $p = 0.006$ ), with HHV-6B DNAemia being associated with increased odds of HCMV DNAemia, controlled for HIV status, age and WFAZ score (OR 2.20 [95%CI 1.36-4.62],  $p = 0.003$ )(Data not shown).

### **Viral Loads**

Comparing viral loads, as defined by 'betaherpesvirus genome equivalents per microgram of extracted DNA', we found the highest viral loads for HHV-7 (Median:13218, IQR:5185-25195) followed by HCMV (Median:2993, IQR:991-10630). HHV-6B median viral load was over a log lower than of HCMV (Median:91, IQR:42-192)(Table 5 and Figure 2a). The single HHV-6A positive sample had a relatively high viral load of 15400 genome equivalents per  $\mu\text{g}$  extracted DNA. Stratifying by HIV status, median viral load for HCMV was significantly higher among HIV-infected children (Figure 2b)(HIV-uninfected: Median 1985 (95%CI 884-6510) vs HIV-infected: Median 3639 (95%CI 1567-18980)( $p = 0.024$ ). Conversely, median HCMV viral load did not differ significantly by weight-for-age z-score (data not shown)( $p = 0.120$ ).

### **Discussion**

Betaherpesvirus DNAemia was detected in two thirds of admitted Zambian infants with HCMV DNAemia (41.3%, 125/303) being roughly twice as prevalent as both HHV-6B (20.5%, 62/303) and HHV-7 (20.1%, 61/303). HCMV DNAemia was twice as prevalent among HIV-infected children consistent with previous studies [4], which have also shown links with AIDS progression [38] and death [14]. The persistent association of HCMV infection with HIV infection and being underweight is consistent with a previous population-based study, which showed that early infant HCMV infection is independently linked with impaired physical development of Zambian children [5].

The median HCMV viral load (3.9 log<sub>10</sub> copies/ml whole blood) was similar to a previous study of hospitalized Zambian children [4]. A recent South African study of HCMV pneumonia in infants used ROC analysis to determine that a viral load of 4.1 log<sub>10</sub> copies/ml whole blood could isolate 70% of cases with a positive urine culture [8]. Here we also analyzed 'copies/μg extracted DNA' (normalizing for the amount of cell DNA present in the specimen) and found that whilst the range of viral loads was extremely broad, median HCMV viral load (copies/μg extracted DNA) was significantly higher (by 1 log) among HIV infected children, an effect not evident for HHV-6 or HHV-7.

HCMV is an established co-factor linked with AIDS progression and neurological sequelae [38] and so the association of HCMV infection with an admission diagnosis of meningitis is interesting and raises the question of to what degree HCMV is causing meningitis in African children, compared with bacterial pathogens, and whether it should be diagnosed and treated? A previous study also found that among HIV-exposed children (those who remain HIV negative but who have HIV positive mothers), HCMV was linked with impaired psychomotor development [5]. Other reports from sub-Saharan Africa have identified both HCMV and HHV-6 among other viral infections as important causes of hospitalization and mortality among children with non-malarial CNS infections [30, 39]. Studies have shown that detection of HCMV DNA in the CSF of patients with suspected meningitis is associated with mortality in both adults [40, 41] and children [42].

With respect to HHV-6B our findings are consistent with a large study from the U.S that demonstrated active HHV-6 infection in 20% of infants admitted with febrile illness [22]. Previous studies from Zambia with smaller sample sizes have detected HHV-6 DNA in the whole blood of 5-30% of children admitted with febrile illness [3, 43]. Classical sequencing over a hypervariable region within the U47 locus identified HHV-6A in 37.5-57% of those that were successfully sequenced [3, 43]. This indication that HHV-6A might be a prevalent infant infection in Africa was followed up by a population-based study of healthy children, which identified HHV-6A in 99% (55/56)(of which 7 cases were co-infected with HHV-6B) and HHV-6B in



15% (8/56) of positives [3]. In light of these previous findings we were surprised to find such a low prevalence of HHV-6A. We detected HHV-6A and HHV-6B using a highly sensitive and specific multiplex Taqman-based Real Time PCR assay with conserved primers but species-specific probes targeting the HHV-6 DNA polymerase gene (U38) [7]. In co-infected patients, the use of conserved primers may result in the masking of the species with lower copy number, but this alone is unlikely to explain the stark contrast between the two studies. There is some limited evidence within the U47 gene for isolates containing both HHV-6A and HHV-6B elements [3, 44], although globally, complete genomic sequences for HHV-6 have only been published for four strains: [45-52]. HHV-6 is integrated into the telomeric repeats of roughly 1% of European and 0.2% of Japanese populations, typically characterized by high loads of HHV-6 DNA in clinical samples [53]. None of the HHV-6-positive samples on this study displayed viral loads (5 log/ $\mu$ g DNA) that might be consistent with chromosomal integration [54].

The distribution of betaherpesvirus prevalence by age, showed profiles indicative of early primary infection with HCMV and HHV-6B peaking between 3-6 months of age, followed by secondary infection or re-activation in children older than 12 months of age. For HCMV this is consistent with previous African studies [5] but different from high-income populations where HCMV infection is later [55]. For HHV-6B our data is consistent with a U.S study [22]. The distribution of HHV-7 infections was distinct from HCMV and HHV-6B, with most primary infections appearing to occur in young neonates, followed by a steady increase in probable reactivated infections during the first year of life, and a very stable prevalence of around 20% among admitted children older than 12 months of age. We present the first data on HHV-7 infections in African infants and the age distribution is consistent with serological data from Brazil [56], yet contrary to U.S data which suggests primary infection with HHV-7 peaks later than HHV-6B [57].

There were several limitations to this study: Analysis of a single sera prevents detailed determination of the clinical and virological course of individual betaherpesvirus infections. The diagnostic tests used cannot accurately differentiate between active infections causal of pathology, and sub-clinical DNAemia arising from reactivated infections secondary to other infections and diseases. Similarly, in the absence of serological data we cannot confirm whether individual infections were primary or secondary, although we could infer some indication of the presence of primary and secondary infections from the age distribution. The use of commercial assays would have greatly strengthened comparisons of prevalence and viral loads with other studies. We did not have access to maternal HIV status preventing comparison of the relevance of HCMV infections in HIV-exposed children, seen in previous studies [5], and could not determine how many of the infections were acquired in utero We did not have infant height data and so could not

calculate z-scores for stunting and wasting, which are more accurate markers of growth delay than underweight (weight-for-age z-score).

Despite these limitations, our findings add weight to previous studies which demonstrate that HCMV infections (both congenital [15, 16, 19] and infant infections [5, 8, 14, 28, 29]) are emerging as important determinants of health and development in African children.

## **LIST OF ABBREVIATIONS**

HCMV - human cytomegalovirus

HHV-6A – human herpesvirus 6A

HHV-6B – human herpesvirus 6B

HHV-7 – human herpesvirus 7

HIV – human immunodeficiency virus

TB - Tuberculosis

CNS – central nervous system

UTH – University Teaching Hospital

PCR – polymerase chain reaction

## **NOTES**

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**Table 1: Oligonucleotide sequences and thermocycling conditions**

Target	Primers and Probes 5'-3'	Cycling Conditions
HCMV UL83[37]	Forward Primer CAGTCCCGAGACMG TGAGAC Reverse Primer TGAACATCCCCAGCATCAACG Probe: FAMb-TGCCACATCTGCTTGCCCGACGC-BBQb	Hold @ 95°C, 10 min Cycling (45 repeats) Step 1 @ 95°C, hold 10 secs Step 2 @ 58°C, hold 20 secs Step 3 @ 72°C, hold 1 secs
HHV-6A and HHV-6B[7]	Forward primer GGAGTGCCTGTGGGTATTC Reverse primer CTAAGGTGAGCCAGATTCTG HHV6A Probe: HEX-TGCAGCCATTTCTTTGGAAAGC-TAMRA HHV6B probe: FAM-TGCAGCCACCTCCTTGAAAG-TAMRA	Hold @ 94°C, 5 min Cycling (55 repeats) Step 1 @ 94°C, hold 15 secs Step 2 @ 60°C, hold 60 secs
HHV-7[36]	Forward Primer TTTCCTGTGACAAAAGAAGCAGTTA Reverse Primer ATCCACACGCTTTACGGG Probe: FAM-TTCCTGCGCAATAAAGTGAAAAGTGTAGCATT-TAMRA	Hold @ 50°C, 2 min Cycling (60 repeats) Step 1 @ 95°C, hold 20 secs Step 2 @ 60°C, hold 60 secs
$\beta$ -actin	Forward Primer: CACACTGTGCCCATCTACGA Reverse Primer: CTCAGTGAGGATCTTCATGAGGTAGT Probe: FAM-ATGCCCTCCCCATGCCATCCTGCGT-TAMRA	Hold 94°C 3 min Cycling (45 cycles) Step 1 @ 94°C Step 2 @ 65°C hold 50 secs

**Table 2: Comparison of key descriptive variables between the study group and admitted population**

	<b>Study Group</b>	<b>Admitted population</b>
<b>n =</b>	303	1176
<b>Dates</b>	Jun 2011 – Apr 2012	Apr-May 2011
<b>Female Gender</b>	128/301 (42.5%)	532/1167 (44.8%)
<b>Median Age (IQR)</b>	12 (7-17) months <sup>a</sup>	8 (3-13) months <sup>a</sup>
<b>Infant HIV infection</b>	101/290 (34.8%) <sup>b</sup>	147/1142 (12.9%) <sup>b</sup>
<b>Mortality</b>	ND	128/1171 (10.9%)
<b>Malnutrition noted on admission</b>	110/303 (36.3%) <sup>c</sup>	117/1176 (9.9%) <sup>c</sup>

Where denominators differ from total sample size this represents missing data for that variable.

Significance was by Pearson chi-squared for binomial variables, by Mann Whitney U test for age and student t test for birth weight.

<sup>a, b, c</sup> p < 0.001, ND = No Data

**Table 3: Betaherpesvirus prevalence stratified by HIV status**

	Unweighted (%. SE)	Weighted <sup>a</sup> (%. SE)	HIV uninfected <sup>b</sup> (%. SE)	HIV infected <sup>b</sup> (%. SE)	p
<b>Any betaherpesvirus</b>	179/303 (59.1, 2.8)	170/303 (56.0, 2.9)	105/191 (55, 3.6)	68/101 (67.3, 4.7)	0.041
<b>Totals</b>					
<b>HCMV</b>	125/303 (41.3, 2.8) <sup>c,d</sup>	111/303 (36.5, 2.8)	65/191 (34, 3.4)	55/101 (54.5, 5.0)	0.001
<b>HHV-6A</b>	1/303 (0.3, 0.3)	2/303 (0.6, 0.4)	1/191 (0.5, 0.5)	0/101 (0%)	0.466
<b>HHV-6B</b>	62/303 (20.5, 2.3) <sup>c</sup>	73/303 (24.0, 2.5)	44/191 (23, 3.1)	17/101 (16.8, 3.7)	0.215
<b>HHV-7</b>	61/303 (20.1, 2.3) <sup>d</sup>	53/303 (17.4, 2.2)	42/191 (22, 3.0)	17/101 (16.8, 3.7)	0.296
<b>Co-infections</b>					
<b>HCMV + HHV-6B</b>	27/303 (8.9, 1.6)	33/303 (10.8, 1.8)	18/191 (9.4, 2.1)	8/101 (7.9, 2.7)	0.668
<b>HCMV + HHV-7</b>	16/303 (5.3, 1.3)	9/303 (2.9, 1.0)	11/191 (5.8, 1.7)	5/101 (5.0, 2.2)	0.773
<b>HHV-6B + HHV-7</b>	9/303 (3, 0.9)	7/303 (2.4, 0.9)	7/191 (3.7, 1.4)	2/101 (2.0, 1.4)	0.428
<b>HCMV + HHV-6B + HHV-7</b>	8/303 (2.6, 0.9)	8/303 (2.4, 0.9)	5/191 (2.6, 1.2)	3/101 (3.0, 1.7)	0.861
<b>HCMV + HHV-6A + HHV-7</b>	1/303 (0.3, 0.3)	2/303 (0.6, 0.5)	1/191 (0.5, 0.5)	0/101 (0, 0)	0.466

<sup>a</sup> Weighted for recruitment biases in age, infant HIV status and malnutrition status on admission

<sup>b</sup> Unweighted data stratified by HIV status

<sup>c,d</sup>  $p < 0.001$

Significance is by Pearson chi squared

**Table 4: Multivariate binary logistic regression analysis of risk factors associated with betaherpesvirus DNAemia in pediatric admissions <2yrs of age**

	HCMV (>200 copies/ml sera)		HHV-6B (>200 copies/ml sera)		HHV-7	
	aOR [95% CI]	p =	aOR [95% CI]	p =	aOR [95% CI]	p =
Male gender	1.12 [0.68-1.85]	0.654	0.59 [0.34-1.06]	0.076	0.69 [0.38-1.27]	0.689
HIV Infected	2.31 [1.37-3.90]	0.002	0.74 [0.39-1.41]	0.361	0.60 [0.31-1.15]	0.125
Fever (>= 38°C)	1.01 [0.47-2.19]	0.976	0.54 [0.18-1.63]	0.277	1.02 [0.41-2.53]	0.963
Underweight (WFAZ < -2)	1.82 [1.06-3.12]	0.030	0.92 [0.50-1.71]	0.791	1.01 [0.55-1.88]	0.967
Age (months)	0.95 [0.91-0.99]	0.012	0.96 [0.92-1.01]	0.080	1.04 [1.0-1.09]	0.064
Breast Fed	2.53 [0.72-8.87]	0.147	3.59 [0.45-28.9]	0.230	2.39 [0.30-19.2]	0.414
Rash in the past month	2.27 [0.77-6.68]	0.136	1.56 [0.44-5.57]	0.495	0.61 [0.22-1.70]	0.345
Admissions Diagnosis						
Pneumonia	0.74 [0.45-1.24]	0.251	0.97 [0.53-1.76]	0.913	1.39 [0.76-2.53]	0.281
Acute Diarrhoea + Dehydration	1.17 [0.53-2.60]	0.701	0.73 [0.26-2.03]	0.549	1.73 [0.71-4.19]	0.846
Malaria	0.66 [0.17-2.58]	0.546	1.34 [0.34-5.32]	0.675	0.35 [0.40-2.81]	0.323
Meningitis <sup>a</sup>	5.72 [1.07-30.5]	0.041	0.40 [0.50-3.36]	0.396	NC	NC

<sup>a</sup>Of 8 children admitted with suspected meningitis, 6 were positive for HCMV, 1 was positive for HHV-6B and none were positive for HHV-7

aOR = adjusted odds ratio. Analysis of all three viruses was adjusted for the effects of age, infant HIV status and being underweight, as defined by having a weight-for-age z-score (WFAZ) < -2.

NC = not calculable

NB. Odds of betaherpesvirus infections were unaffected by infant TB status

**Table 5: Betaherpesvirus loads**

	Genome equivalents/μg extracted DNA				Genome equivalents/ml whole blood			
	Median	IQR	Min	Max	Median	IQR	Min	Max
HCMV	2993 <sup>a,b</sup>	991-10630	31	207262671	9404	3739-38640	207	775162391
HHV-6A	15400	15400-15400	15400	15400	172812	172812-172812	172812	172812
HHV-6B	91 <sup>a,c</sup>	42-192	2	27389	498	296-1062	201	27115
HHV-7	13218 <sup>b,c</sup>	5185-25195	247	5267277	56868	23772-140704	4005	9547567

<sup>a,b,c</sup> p < 0.001 Mann Whitney U

Figure 1a: Betaherpesvirus prevalence stratified by age among admitted neonates <2 years of age

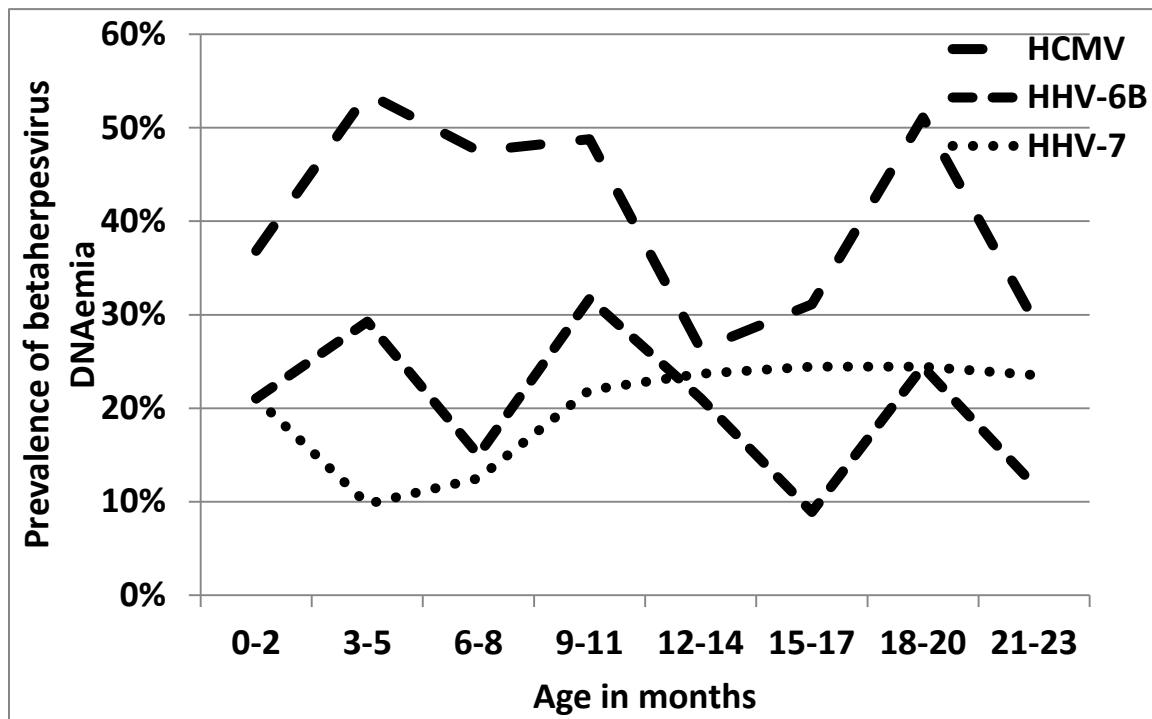


Figure 1b: HCMV prevalence stratified by age and HIV status among admitted infants

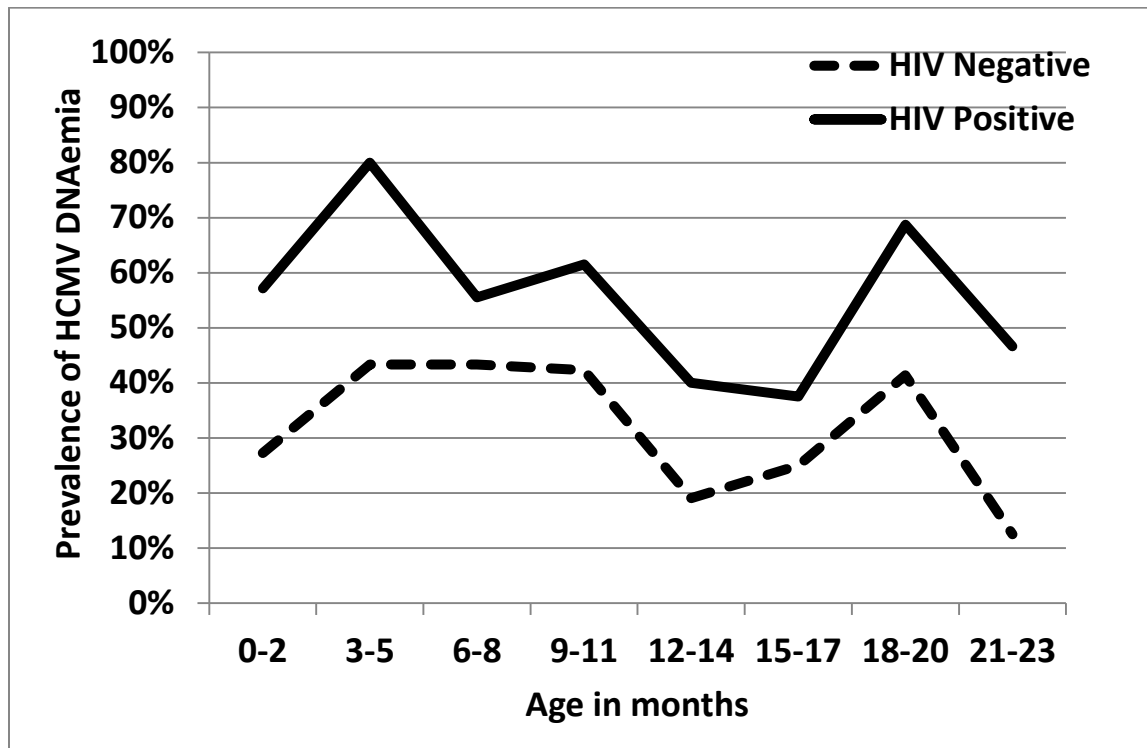


Figure 1c: HCMV prevalence stratified by age and underweight among admitted infants

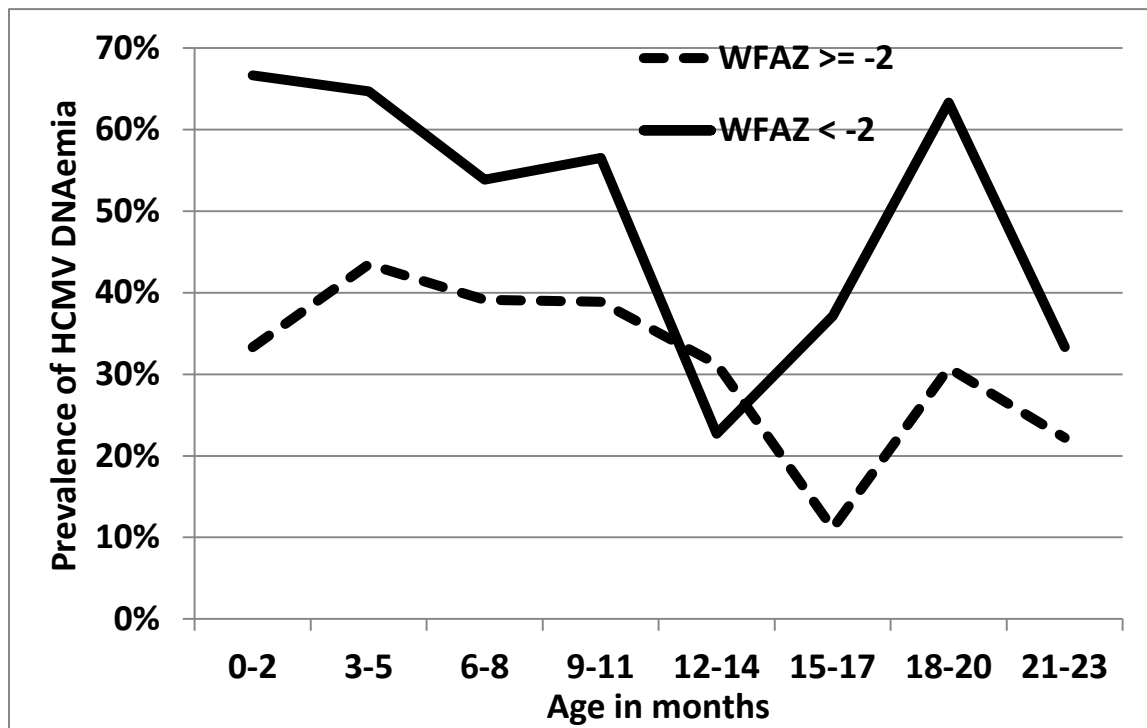
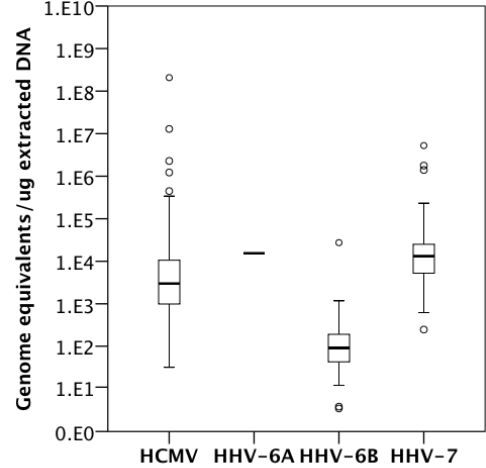
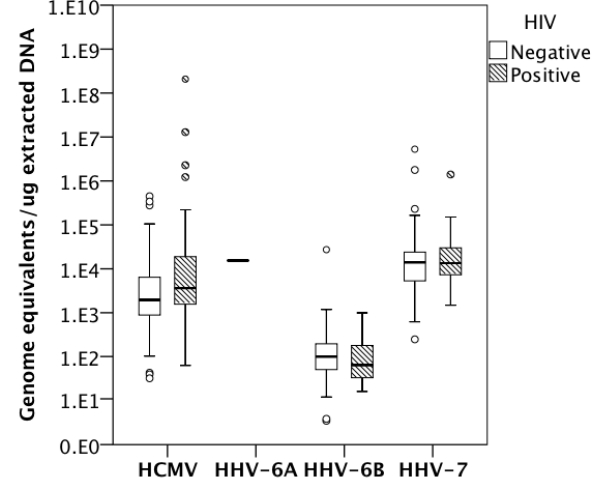


Figure 2A: Betaherpesvirus genomes copies/μg extracted DNA



Boxes indicate interquartile range, whiskers indicate 5<sup>th</sup> and 95<sup>th</sup> percentiles. Circles represent outliers.

Figure 2B: Betaherpesvirus genome copies stratified by HIV status



Boxes indicate interquartile range, whiskers indicate 5<sup>th</sup> and 95<sup>th</sup> percentiles. Circles represent outliers.